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Exercise induced arterial desaturation in recreationally active males at moderate altitude helps explain variability in relationship between total hemoglobin mass and $VO_{2\max}$

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Defense Date: October 31st, 2014

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Abstract

Exercise induced arterial desaturation (EIAD) occurs in a large percentage of highly trained athletes at sea-level (SL), but is extremely rare in less trained populations at SL. Altitude can increase the risk for EIAD, but little research has looked at the prevalence of EIAD in recreationally active males at moderate altitude (MA). Moreover, previous research has identified a strong correlation between total hemoglobin mass (tHb) and maximal oxygen uptake (VO_{2max}). However, no research has looked at the effect that EIAD might have on the VO_{2max} -tHb relationship. The current study sought to determine the prevalence of EIAD in recreationally active males at moderate altitude, and further to determine how this effected the VO_{2max} -tHb relationship. Additionally, we looked at how tHb and EIAD influenced performance at VO_{2max} . Six recreationally active subjects living at moderate altitude (~1655 m) completed 5 separate visits. This consisted of a graded exercise test to determine VO_{2max} , two endurance performance tasks at peak power output, and two tHb measurements using the optimized carbon monoxide rebreathing procedure. Preliminary results show that four of six subjects experienced EIAD. Additionally, while tHb alone explained a large portion of the variability in VO_{2max} ($\text{adj } r^2_{(\text{rel. } VO_{2max}, \text{ rel. tHb})} = 0.66$; $\text{adj } r^2_{(VO_{2max}, \text{ tHb})} = 0.86$), adding arterial oxygen saturation (S_aO_2) into the model increased this relationship ($\text{adj } R^2_{(\text{rel. } VO_{2max}, \text{ rel. tHb}, S_aO_2)} = 0.72$; $\text{adj } R^2_{(VO_{2max}, \text{ tHb}, S_aO_2)} = 0.90$). No correlation was found between end exercise S_aO_2 , tHb, and performance at VO_{2max} . Our results demonstrate that the conventional model of VO_{2max} -tHb can be improved by taking into account S_aO_2 in populations that experience EIAD. Additionally, they highlight the importance of accounting for S_aO_2 as well as tHb as markers for oxygen transport and whole body oxygen carrying capability in relation to VO_{2max} .

Note on convention: correlations are presented using the adjusted coefficient of determination (discussed in methods section). When representing the adjusted coefficient of determination corresponding to bivariate regression analysis, lowercase symbols are used (ex: $\text{adj } r^2$). When representing the adjusted coefficient of determination corresponding to multivariate regression analysis, uppercase symbols are used (ex: $\text{adj } R^2$). Additionally, subscripts following this symbol display first the criterion measure, followed by any predictor measures used in the model (ex: $\text{adj } R^2_{(\text{criterion measure}, \text{ predictor variable 1}, \text{ predictor variable 2})}$). Finally, all data are reported as the mean \pm 1SD.

Introduction

Humans have long been interested in athletic performance. From the early Olympic games and the first marathon, to more modern athletic achievements such as the 4-minute mile, winning a cycling grand tour, or the yet intangible sub 2 hour marathon, there is something about feats of athletic endurance that have the ability to produce admiration and awe. Since humans place such an extraordinary importance on these exploits, it's no surprise that there is a long history of scientific exploration into the physiological characteristics that underlie these activities. Nine decades ago, contemporary exercise science was born when A.V. Hill defined the concept of $\text{VO}_{2\text{max}}$, the maximal rate of oxygen uptake (Bassett and Howley 2000). Since then, $\text{VO}_{2\text{max}}$ has been the most commonly used indicator of human endurance capacity. However, since the discovery of $\text{VO}_{2\text{max}}$ it has been shown that there are large variations in $\text{VO}_{2\text{max}}$ among athletes of the same ability (Sjodin and Svedenhag 1985, Tanaka 1990). This clearly shows that $\text{VO}_{2\text{max}}$ is not the only important factor for endurance performance. In fact, $\text{VO}_{2\text{max}}$ is only one of a few key parameters for endurance performance, which also include exercise economy and lactate threshold (LT) (Coyle 1994). Exercise economy is defined as the oxygen required to maintain a given velocity (running) or power (cycling), while LT is defined as the workload at which the rate of appearance of lactate in the blood becomes greater than the rate of lactate uptake. Both of these factors are highly correlated with endurance performance (Coyle 1994, Bassett and Howley 2000, Jones and Carter 2000). In competitive endurance athletes where the main goal is to sustain the maximum possible velocity over a given distance, $\text{VO}_{2\text{max}}$, lactate threshold, and exercise economy all function independently to influence race velocity. However, $\text{VO}_{2\text{max}}$ is arguably the most important factor out of these three, because it sets the upper limit for aerobic metabolism, which effectively determines the relative importance of LT and economy on race velocity or power (Joyner and Coyle 2008). The primary goal of this research was to determine the effect that total hemoglobin mass (tHb) and arterial oxygen saturation (S_aO_2) have on $\text{VO}_{2\text{max}}$. In order to understand why we performed this study, we will begin by discussing the factors that determine $\text{VO}_{2\text{max}}$.

In the process of converting biochemical energy into mechanical energy, cellular respiration produces ATP which in working muscle is provided to the cross bridges to perform work. During cellular respiration, there are many pathways that lead to the production of ATP, but during endurance exercise the vast majority of the ATP produced comes from aerobic respiration. In this process, oxygen is used as the final electron acceptor for the mitochondrial electron transport chain, and because of this aerobic respiration effectively stops when oxygen is not present. It is well known that the rate of oxygen delivery to working muscle is one of the limiting factors during both submaximal and maximal endurance exercise (Amann 2012). Further, interventions that alter the rate of oxygen delivery accordingly

increase or decrease the ability of the muscles to do work (Bassett and Howley 2000). Therefore it is important to understand what influences the rate of oxygen delivery. It is traditionally believed that there are four major limiting factors to $\text{VO}_{2\text{max}}$: 1. the ability of the pulmonary system to saturate the blood, 2. maximum cardiac output, 3. the oxygen carrying capacity of the blood, and 4. skeletal muscle limitations (Bassett and Howley 2000). When considered sequentially, this process has been referred to as the oxygen transport cascade (Richardson 1998, Hoppeler and Weibel 1998) and consists of the following steps:

1. The movement of oxygen into the lungs (air→alveoli)
2. The movement of oxygen into the blood (alveoli→arterial)
3. The movement of oxygen to the working muscle (arterial→capillaries→myoglobin), and
4. The chemical reduction of oxygen into water in the muscle (myoglobin→mitochondria).

The movement of oxygen from the external environment to the arterial blood (steps 1 and 2) can be measured by either the arterial partial pressure of oxygen (P_{aO_2}) or the arterial oxygen saturation (S_{aO_2}). Hemoglobin concentration ($[\text{Hb}]$) (along with S_{aO_2}) is the primary factor responsible for the oxygen content per unit blood, while cardiac output determines the volume of blood ejected from the heart per unit time and has been shown to be linearly correlated with leg blood flow during exercise (Mortensen et al. 2005). Ultimately, oxygen diffuses into the working muscle.

By looking at oxygen delivery in terms of the oxygen transport cascade, it is clear that an increase in S_{aO_2} , $[\text{Hb}]$, or cardiac output can all independently affect $\text{VO}_{2\text{max}}$. This model is how $\text{VO}_{2\text{max}}$ is traditionally presented (Bassett and Howley 2000). However, if instead we look at total hemoglobin mass (tHb) (as opposed to $[\text{Hb}]$), the picture becomes slightly different. It has previously been shown that as fitness increases, $[\text{Hb}]$ stays the same or even decreases (Schmidt and Prommer 2010, Jones and Carter 2000), and further that $[\text{Hb}]$ does not correlate well with $\text{VO}_{2\text{max}}$ (Schmidt and Prommer 2010). In general, when looking across the population, as tHb increases so too does cardiorespiratory fitness (Schmidt and Prommer 2010, Jones and Carter 2000). In order for $[\text{Hb}]$ to stay relatively constant (or decrease) as fitness increases, plasma volume must also increase. Increased plasma volume leads to an increase in total blood volume, which in turn results in an increase in maximum cardiac output (Schmidt and Prommer 2010). This shows how tHb is related to both total oxygen carrying capacity as well as cardiac output, and is therefore highly correlated with $\text{VO}_{2\text{max}}$ (Heinicke et al. 2001, Schmidt and Prommer 2010). Additionally, when blood is fully saturated with oxygen, the oxygen carrying capacity of the blood mostly depends on tHb (there is also a small amount of oxygen dissolved in the plasma). However, in some situations S_{aO_2} decreases by a significant amount (Dempsey and Wagner 1999) so that the total oxygen carried in the blood depends on both tHb and

S_aO_2 . Since S_aO_2 is easily manipulated both by environmental as well as physiological conditions, we will now explore the factors that influence this parameter.

Since P_aO_2 is dependent on the rate at which O_2 diffuses into the blood, it is determined primarily by the pulmonary system. This is a complex process that depends on the following factors:

1. The partial pressure of oxygen in the environment
2. The exchange of gasses between the atmosphere and the lung, and
3. The exchange of gasses between the lung and the blood.

Each of these factors can change independently, leading to a highly fluid model that can be difficult to predict. In looking at S_aO_2 changes during exercise, it helps to understand these three factors in greater detail.

1. The percent of oxygen in the environment is a constant 20.9% despite changes in altitude. However, as altitude increases, barometric pressure decreases, leading to a decreased P_aO_2 . This hypoxic environment leads to a decrease in endurance performance (Bassett and Howley 2000).

2. The exchange of gasses between the atmosphere and the lung is an important factor in determining rate of oxygen delivery. Specifically, oxygen must reach the exchange surfaces of the lung (the respiratory bronchioles and alveoli) to be able to diffuse into the blood. For this reason, alveolar ventilation (the amount of gas reaching the exchange surfaces per unit time) is commonly referred to as the most important ventilatory parameter (Di Prampero 2003). Alveolar ventilation depends on tidal volume (the amount of air moved per breathe), breathing frequency, and dead space volume (the volume of air that does not reach the exchange surfaces). During exercise, constriction (or simply lack of dilation) in either intrathoracic airways (as in exercise induced asthma) or extrathoracic airways (as in vocal cord dysfunction) can lead to decreased alveolar partial pressure of oxygen (P_{AO_2}), and subsequently decreases in P_aO_2 and S_aO_2 (Dempsey et al. 2008). Additionally, as workload increases, so too does the demand for oxygen, and as a result, ventilation increases to keep up with oxygen demand. The resulting high ventilation can lead to two issues. First, as ventilation increases so does the work of breathing, and during heavy exercise respiratory muscles can fatigue (Dempsey et al. 2008, Johnson et al. 1992). Respiratory muscle fatigue can hypothetically lead to a relative alveolar hypoventilation, but in practice it has been shown that during exercise, it does not lead to a decrease in P_{AO_2} (Johnson et al. 1992). Second, as trained subjects reach higher ventilations, there is evidence that mechanical constraints of the lung become a limiting factor to increasing ventilation (Dempsey et al. 2008, Johnson et al. 1992). Consequently, in recreationally active people, exercise induced asthma and vocal cord dysfunction can lead to exercise induced desaturation, but mechanical ventilatory constraints and work of breathing rarely influence S_aO_2 in this population (Dempsey et al. 2008).

3. The exchange of gasses between the lung and the blood is the final step in delivering environmental oxygen to the blood. The exchange at each individual alveoli depends on surface area, diffusion distance, partial pressure gradient, and membrane permeability (Silverthorn et al. 2009). Except in uncommon pathological cases such as pulmonary edema which cause an increase in diffusion distance, the primary determinate of gas exchange at each individual alveoli is the partial pressure gradient. The partial pressure gradient depends on P_{AO_2} and P_{aO_2} , and is therefore highly dependent on the environmental partial pressure of oxygen.

Three additional factors that can influence gas exchange and impact some of the factors above are pulmonary capillary transit time, ventilation-perfusion mismatch, and pulmonary or cardiac shunts.

Pulmonary capillary transit time describes the concept that as a single red blood cell passes through the capillary exchange surface, there is a finite and specific amount of time that it has to complete gas exchange. As cardiac output increases, the velocity of each red blood cell through the capillaries increases, which decreases the pulmonary capillary transit time. In highly trained endurance athletes with high cardiac outputs, when exercising at or near VO_{2max} this transit time can become insufficient to complete gas exchange, which may lead to arterial hypoxemia (Dempsey et al. 1984, Powers et al. 1989, Dempsey & Wagner 1999, Prefaut et al. 2000). However, pulmonary capillary transit time is not thought to be a limiting factor to VO_{2max} in less trained subjects at sea level, since such a population generally has smaller cardiac outputs relative to lung size which leads to longer pulmonary capillary transit times.

Ventilation-perfusion mismatch occurs when the alveolar ventilation of a single gas exchange unit is higher than the perfusion of the unit, leading to an excess of oxygen relative to the ability of the blood to carry it. In the orthostatic position, ventilation and perfusion increase from the apex to the base of the lung, but perfusion increases to a much greater degree due to gravity. These changes lead to a situation where the superior aspect of the lung is over-ventilated while the inferior aspect is over-perfused. At rest, ventilation-perfusion mismatch can explain up to 50% of the alveolar-arterial oxygen difference $((A-a)DO_2)$ (Prefaut et al. 2000). During exercise, ventilation-perfusion mismatch has been shown to effect $(A-a)DO_2$ in trained athletes who experience only mild reductions in P_{aO_2} (Hopkins et al. 1994, Prefaut et al. 2000). However the roll of ventilation-perfusion mismatch during exercise is still a topic of debate (Dempsey and Wagner 1999).

A shunt occurs when blood bypasses the capillaries where gas exchange occurs. Shunts can affect the exchange of gas between the lung and the blood, decreasing gas exchange and subsequently lowering P_{aO_2} . Arteriovenous intrapulmonary shunts are thought to be the result of increasing pulmonary vascular pressure as cardiac output climbs during exercise. As cardiac output increases, distinct vascular conduits may open in response to the increased pressure, allowing

preservation of cardiac output without damaging the thin, delicate alveolar tissues. Arteriovenous intrapulmonary shunts may contribute to the decreased S_aO_2 observed during exercise, and have been shown to be present in a majority of the population (Eldridge et al. 2004). In addition, there are multiple different cardiac defects that can result in a shunt between the left and right sides of the heart. One example is the patent foramen ovale, which is an opening between the left and right atrium. This congenital heart defect is fairly common, appearing in 20-30 percent of the population, but is usually asymptomatic and therefore goes undiagnosed (Kaplan 1993). During exercise, intracardiac shunts can increase $(A-a)DO_2$, leading to a decreased exercise P_aO_2 in this portion of the population.

As shown above, there are many issues that can decrease P_aO_2 during both submaximal and maximal exercise. When a decrease occurs, the resulting phenomenon is known as exercise induced arterial hypoxemia (EIAH) (Dempsey et al. 1984, Powers et al. 1989, Dempsey & Wagner 1999, Prefaut et al. 2000). Although in the most rigid sense EIAH is defined as a decrease in P_aO_2 due directly to exercise, it has also previously been defined as a decrease in arterial oxygen saturation (S_aO_2) (Dempsey and Wagner 1999). For our purposes, EIAH will refer solely to a decrease in P_aO_2 during exercise, while exercise induced desaturation (EIAD) will refer to a decrease in S_aO_2 during exercise. Importantly, while these conditions are not necessarily mutually exclusive, they are distinct phenomena. S_aO_2 depends not only on P_aO_2 , but also on the affinity of oxygen to hemoglobin, which changes based on factors such as the temperature and pH of the blood. This relationship is known as the oxyhemoglobin dissociation curve and relates partial pressure of O_2 and the percent saturation of hemoglobin. Finally, because only a tiny portion of oxygen is carried directly dissolved in the blood, changes in S_aO_2 are much more likely to impact endurance performance.

Previous research has shown that tHb is highly correlated with VO_{2max} at sea level (Heinicke et al. 2001, Schmidt and Prommer 2010), but no research has looked at how S_aO_2 influences the VO_{2max} -tHb relationship. Additionally, athletes with a higher VO_{2max} have a higher chance of experiencing a significant decrease in S_aO_2 during exercise (Dempsey and Wagner 1999). Since the reduction in S_aO_2 becomes more pronounced as elevation increases (Dempsey and Wagner 1999), EIAD is expected to be exacerbated at moderate altitudes like Boulder (~5430 feet; ~1655 m). Despite the high degree of correlation between tHb and VO_{2max} , little research has examined the relationships between these parameters at moderate altitude. Also, to our knowledge no one has looked at the prevalence of EIAD at moderate altitude in recreationally active males. We believe that by adding the measurement of S_aO_2 into the current VO_{2max} -tHb model, we will better represent whole body oxygen carrying capability, leading to an even stronger correlation with VO_{2max} . The primary goal of the current research was to describe how these variables interact in recreationally active males at moderate altitude. To make this assessment, data was collected on tHb, VO_{2max} , time to exhaustion at peak power output (TTE), and

exercise induced desaturation during the exercise tests. We had three hypotheses. First, we hypothesized that EIAD would be observed in acclimatized recreationally active athletes at moderate altitude who we would not typically expect to develop EIAD at sea level, and that subjects with higher VO_{2max} would have higher prevalence of EIAD. Second, we hypothesized that tHb and end exercise S_aO_2 would result in a stronger relationship with VO_{2max} than tHb alone. Third, we hypothesized that tHb and S_aO_2 would correlate with the endurance performance task (TTE), which was performed at a power meant to elicit VO_{2max} .

Methods

This project was conducted as part of a larger project investigating the effects of low dose, chronic, intermittent carbon monoxide (CO) inhalation on various hematological and endurance performance parameters in recreationally active males. Institutional Review Board (IRB) approval for this project was granted through the University of Colorado IRB, and all subjects gave written informed consent. To investigate the hypotheses given above, only data from the baseline testing was used, resulting in a total of five visits. This consisted of one graded exercise test, two total hemoglobin tests, and two time to exhaustion tests. Prior to all three exercise tests, subjects were asked maintain a food diary for the 24 hours leading up to the test. This requirement helped to ensure that subjects arrived in the same postprandial state. Subjects were also asked to arrive euhydrated and to pay particular attention to caffeine consumption in an attempt to maintain hydration and caffeine status between tests. Finally, subjects were asked to get adequate sleep and abstain from vigorous exercise the day prior to the performance tests. Testing was performed in the following format:

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
<i>Visit 1</i>		<i>Visit 2</i>	<i>Visit 3</i>		<i>Visit 4</i>	<i>Visit 5</i>
Consent Form Blood Draw GXT		Total Hemoglobin Mass	Time To Exhaustion		Total Hemoglobin Mass	Time To Exhaustion

Subjects

Eight healthy recreationally active males with an average age of 24.5 ± 3.3 years completed the study as of this writing. Average height of the subjects was 179.5 ± 7.7 cm, body mass was 76.3 ± 10.8 kg, and BMI was 23.5 ± 1.9 kg/m². Two subjects did not have S_aO_2 values measured. Recreationally active was defined as no more than 30 minutes of exercise, five days per week, which corresponds to the American College of Sports Medicine's minimum recommendations for cardiorespiratory exercise

(Garber et al. 2011). This was important, as we expected a different response from recreationally active versus highly trained groups. Finally, all subjects resided at moderate altitude for a minimum of 8 weeks prior to the start of the study.

Visit 1

Potential subjects were scheduled for an initial visit after they were adequately screened for the study. On day one, they completed a questionnaire to assess their ability to safely complete the high intensity exercise tests. Following this questionnaire, the subject's body weight and height were recorded. After this, a venous blood draw was performed to evaluate initial ferritin levels. Ferritin levels were checked to ensure that none of the subjects were iron deficient (ferritin < 20 ng/dL), which could have confounded our findings (Garza et al. 1997). Once the blood sample was collected, the subject began the graded exercise test (GXT) to determine $\text{VO}_{2\text{max}}$, change in S_aO_2 , LT, and economy. The GXT was performed using a stationary bicycle (Lode ergometer, Netherlands), a metabolic cart to perform indirect calorimetry (Parvomedics Truemax 2400, USA), and a forehead pulse oximeter (Nellcor N-595, USA) to measure S_aO_2 . Forehead pulse oximetry has been shown to be a stable and reliable measure of S_aO_2 during exercise (Yamaya et al. 2002). After the subject was fit to the bicycle and the forehead pulse oximeter was connected, a commercially available hand warmer was placed in a nitrile glove with the finger tips removed. This glove was fitted to the subject in order to adequately arterialize the capillary blood in preparation for lactate sampling. Once the subject had been seated on the bike for ~5 minutes in order to allow stabilization, baseline saturation and lactate were collected. A ~25 μL blood sample was obtained from a pre-warmed fingertip, and was processed using a YSI 2300 Blood glucose/Lactate analyzer (YSI, USA). Following this, the subject began breathing through the mouthpiece of the metabolic cart, and the GXT began. During the GXT, the workload began at 50 Watts and increased in increments of 30 Watts every four minutes until the subject reached volitional fatigue. Between minutes three and four of each stage, the subject was asked to report their rating of perceived exertion (RPE) on a scale of 6-20 (Borg 1982) and an additional lactate sample was collected. Arterial oxygen saturation was recorded every 15 seconds during the final minutes of exercise until the subject reached volitional exhaustion, at which point the GXT was terminated. The average S_aO_2 over the final minute of the GXT was considered to be the end GXT S_aO_2 , and subjects were considered to have experienced EIAD if their oxygen saturation dropped more than 3% from baseline (Dempsey and Wagner 1999). LT was defined as the point where blood lactate increased by 1 mMol above baseline values (Coyle 1994), while economy was defined as the power output at LT divided by the absolute steady state oxygen consumption VO_2 at this point. Time adjusted PPO was determined by calculating the percentage of the final stage successfully accomplished, multiplying by

30 Watts (equal to the change in power output per stage), and adding this to the power output of the final stage completed in full (Brothers et al. 2007).

Visits 2 and 4

On visits 2 and 4, subjects completed a total hemoglobin mass measurement using the optimized CO rebreathing method (oCO_r) as described by Schmidt and Prommer (2005). This method involves having the subject inhale a small, known amount of CO and measuring the change in percent carboxyhemoglobin. The subject began by sitting for ~10 minutes in order to allow the vascular volumes to stabilize. During this time their hand was placed in a heating pad to arterialize fingertip capillary blood. Following this, a fingerstick was performed in order to collect two ~75 µL blood samples, which were used to assess baseline carboxyhemoglobin levels (OSM3 Hemoximeter, Radiometer, Denmark). The subject then performed a full exhalation to residual volume into a portable CO detector (Draeger Pac 7000, Draeger, Germany) in order to quantify baseline end tidal CO concentration. Following this, the subject then performed the CO rebreathing portion of the test. Initially, subjects were shown a video to acquaint them with the test. During the actual procedure, a custom spirometer (Spico-CO Respirations-Applikator, Blood Tec, Germany) was used. The majority of the gas in the spirometer (~3 liters) is pure oxygen, while a small portion of 99.5% CO (1.2 mL per kg body weight) is administered. The subject began the rebreathing procedure by placing a nose clip. Following this, the subject brought their lips up to the mouthpiece while exhaling down to residual volume. Once residual volume was reached, the subject signaled the investigators. At this point, the valves were opened, and the subject took a deep breath in (consisting of all the CO as well as a portion of the oxygen) and held it for 10 seconds. Following this, the subject breathed normally for 1 minute and 50 seconds, ending with another maximal forced exhalation into the spirometer. The gas remaining in the spirometer was measured using a portable CO detector as well as a calibrated 3 liter syringe to determine the volume of CO remaining. Following the rebreathing procedure, the subject was instructed to place their hand back into the heating pad. Four minutes after beginning the procedure, the subject performed another maximal forced exhalation to determine post-test end tidal [CO]. Additionally, two final ~75 µL blood samples were collected 7 minutes after the start of the CO rebreathing procedure which were used to determine post-test carboxyhemoglobin levels. This data was then analyzed using previously published formulas (Schmidt and Prommer 2005, Prommer and Schmidt 2007). It has been shown that taking duplicate measures reduces typical error of tHb measurement by $\sqrt{2}$ (Garvican et al. 2011), and so these measures were averaged to get a better representation of each subject's true tHb.

Visits 3 and 5

On visits 3 and 5, the subject underwent a time to exhaustion (TTE) test at peak power output (PPO). The subject began by warming up at an intensity equal to 1 Watt/kilogram of body weight for 10 minutes. Following the warmup, the subject cycled at workload equal to the PPO from the GXT. During this test, the subject attempted to cycle as long as possible. Once the subject reached volitional exhaustion, the time was recorded. TTE has been shown to be a reliable measure as it is highly sensitive to differences in endurance between subjects (Amann et al. 2008). During the endurance performance test, S_aO_2 was measured as described previously.

Data Analysis

Tests performed in duplicate (TTE, tHb, and EIAD during the TTE) were assessed for normality using the Anderson-Darling test statistic and homogeneity of variance using the dependent t-test before using the dependent t-test to test for differences in the mean (MVPstats). Once the means were determined to be equivalent, the 1st and 2nd tests were averaged. Then, single and multiple regression analysis were performed on the average values using SPSS. Stepwise multiple regression was performed in order to assess whether adding the additional predictor variable end GXT S_aO_2 increased the coefficient of determination above and beyond what would be expected from tHb alone. Additionally, even though we report the correlation coefficient (R) and the coefficient of determination (R^2), we believe that the most important value for our analysis is the adjusted coefficient of determination (adj R^2). When performing regression analysis, adding more independent variables has the tendency to increase the coefficient of determination regardless of whether they actually explain additional variability in the model. The adjusted coefficient of determination takes this into consideration by accounting for the number of independent variables in the model as well as the number of data points. In other words, while R^2 is a sample statistic computed from the data, adj R^2 is an unbiased estimate of the population parameter, which is ultimately what we are interested in. Usually R^2 and adj R^2 will be fairly close. However for our analysis since we have 3 variables and only 6 data points, using adj R^2 is prudent.

Results

Subject Characteristics

Average endurance parameters for our subjects are shown in Table 1. Average relative VO_{2max} was equal to $49.8 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with a range from 41 to $59 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. This is a fairly large range of values, representing a spread from moderate (~35th percentile) to high (~95th percentile) cardiovascular fitness for males of this age group (Sanders and Duncan 2006, American College of Sports Medicine

2013). Average time to exhaustion during the endurance performance task was 500 ± 123 seconds ($8:20 \pm 2:03$).

Total Hemoglobin Mass

Total hemoglobin mass data can be found in Table 2, and matches with expected values for this population. The average tHb for our subjects was 954.5 ± 123.5 grams. Moreover, we observed a large range for tHb (789 to 1135 grams). Relative tHb likewise showed large variability, with an average of 12.6 ± 1.1 g/kg, and an individual range from 11.5 to 14.4 g/kg. During data collection, we measured tHb in duplicate. However, for two separate subjects during one of the rebreathing procedures, we measured a significant CO leak in the equipment. Since it has previously been shown that major leaks significantly increase the measurement error during the oCO_r method and result in artificial inflation of the individual test (Ryan et al. 2011), for these two subjects the lowest measured tHb was used instead of averaging both tests.

Saturation

Resting saturation values (Table 2) for the GXT and TTE were on the average within 2% of maximum. However there was quite a large intra-subject variability (Figure 1). In fact, during rest before the GXT one subject was below the S_aO₂ criteria for mild EIAH as proposed by Dempsey and Wagner (1999), which is one reason that we chose to define EIAD as a 3% or greater decrease in S_aO₂ during exercise. Subjects also showed a large variability in desaturation (GXT: $3.7 \pm 1.6\%$, range 1.45 – 6%. TTE: $3.9 \pm 1.3\%$, range 2.75 – 6%) (Figure 2). Ultimately, we found that ~67% of our subjects experienced either mild (>3%) or moderate (>4%) EIAD over 3 separate exercise trials (Figure 2).

Correlation

Since our main goal was to assess whether correlation of VO_{2max} and tHb was improved by adding end exercise S_aO₂, and since S_aO₂ was only collected in 6 subjects, all correlations were performed using n=6. Although this is a very small sample size, these are only preliminary results, and the ultimate goal is to perform the same analysis with 16 subjects.

First, we found significant correlations between tHb and VO_{2max}, both for absolute and relative measures (mL*min⁻¹ vs. g, and mL*min⁻¹*kg⁻¹ vs. g*kg⁻¹ respectively). As has been previously reported, the correlation was higher for absolute (adj r²_(VO2max, tHb) = .856) versus relative measures (adj r²_(rel VO2max, rel. tHb) = .656) (Schmidt and Prommer 2010). The calculated slope for absolute and relative measures were determined to be 3.56 and 3.48 mL*min⁻¹*g⁻¹, respectively (Figure 3).

Additionally, we found that by adding end GXT S_aO_2 to the model, adj R^2 increased for both the absolute and relative models. For VO_{2max} and tHb, adj $r^2_{(VO_{2max}, tHb)} = .856$, but by adding end GXT S_aO_2 the adj R^2 rose to .900. For relative VO_{2max} and relative tHb, adding end GXT S_aO_2 increased the adj R^2 from .657 to .718 (Table 3, Figure 4). However, we found that with our current sample size, the change in the coefficient of determination after adding S_aO_2 (ΔR^2) was not significant ($p = 0.196$ for $\Delta R^2_{(absolute)}$; $p = 0.265$ for $\Delta R^2_{(relative)}$, Table 3). However, assuming that the correlation stays the same as we obtain more subjects, simulations show that at a sample size of $n = 12$, S_aO_2 will provide a statistically significant effect to the overall VO_{2max} -tHb model for both absolute and relative measures.

Finally, there was no significant correlation between VO_{2max} (either absolute or relative) and EIAD during the GXT. Also, there was no significant correlation between tHb, end exercise S_aO_2 , and TTE at PPO.

Discussion

The primary findings of this investigation are threefold. First, EIAD was observed in a majority of recreationally active subjects at moderate altitude. Second, we showed that adding end GXT S_aO_2 to tHb increases the correlation with VO_{2max} , which is to our knowledge the first evidence that S_aO_2 interacts with tHb to influence VO_{2max} . Third, we found no correlation between tHb, end exercise S_aO_2 , and performance at VO_{2max} .

Exercise induced arterial desaturation

Previous research has shown no discernable occurrence of EIAD in either untrained ($VO_{2max} = 46 \pm 2.8 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) or moderately trained ($VO_{2max} = 55.3 \pm 2.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) subjects at sea level (Powers et al. 1988). However, at moderate altitude we have shown that over half of the moderately trained population may be expected to experience EIAD. While our subjects fit into the category of moderate to high cardiovascular fitness, importantly they did not qualify as elite or highly trained based on their VO_{2max} (Brothers et al. 2007, Sjodin and Svedenhag 1985, Coyle 1994). Additional metrics such as PPO, absolute VO_{2max} at LT, and percent VO_{2max} at LT confirm that our subjects were not highly endurance trained when compared to endurance trained cyclists at moderate altitude (PPO: 258.9 ± 29.8 versus 375.08 ± 22.98 Watts, absolute VO_{2max} at LT: 2.4 ± 0.35 versus $3.7 \pm 0.28 \text{ L/min}$, % VO_{2max} at LT: 64 ± 5 versus $80.3 \pm 5.2\%$) (Brothers et al. 2007). Additionally, we found that VO_{2max} was not significantly correlated with GXT or TTE desaturation. It is quite possible that as our sample size increases this will change. However, as it stands higher aerobic fitness does not appear to be a major risk factor for EIAD within this population. In contrast to previous reports that mainly attribute EIAD in healthy males to being highly endurance trained, we present the novel finding

that at moderate altitude, EIAD occurs in a majority of healthy, young, untrained acclimatized males. This finding is almost certainly due to the decreased partial pressure of oxygen in the environment. However, given the current protocol design, we cannot determine how the decreased environmental partial pressure of oxygen affects specific contributing factors to EIAD (as discussed in the introduction), and so further research is needed to determine how these factors change based on changes in altitude.

Effects of exercise induced arterial desaturation and tHb on VO_{2max} and Performance

Previous research has shown that tHb and VO_{2max} are highly correlated at sea level (Schmidt and Prommer 2010), and our results at moderate altitude support this finding. The coefficient of determination between absolute tHb and absolute VO_{2max} has been reported previously as $r^2 = 0.94$, with a slope of $4.4 \text{ mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ (Schmidt and Prommer 2010). Our findings were largely compatible with these findings. Between absolute tHb and absolute VO_{2max} , we found an adj $r^2_{(VO_{2max}, tHb)} = 0.856$ and a slope of $3.6 \text{ mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$. Since Schmidt and Prommer (2010) had a fairly large sample size, they were able to make predictive claims based on their regression analysis. Ultimately, they came to the conclusion that across the population, an increase of 1 gram in tHb causes a change in VO_{2max} by $\sim 4 \text{ mL} \cdot \text{min}^{-1}$ (Schmidt and Prommer 2010). Our results support this finding, since our data can be interpreted as showing that a 1 gram increase in tHb causes a change of $3.6 \text{ mL} \cdot \text{min}^{-1}$. Although this value is slightly lower than the value reported by Schmidt and Prommer, 99% confidence intervals for our data overlap with the reported values, indicating that our findings are not significantly different. Although our sample size and protocol design prevent us from making predictive claims, it is important to know that our subjects fit relatively well into previously established models for VO_{2max} and tHb.

An important finding of this research is that by adding end exercise S_aO_2 to the model of tHb and VO_{2max} , the adjusted coefficient of determination increases (Table 3, Figures 3, 4, 5, 6). For relative VO_{2max} , adding S_aO_2 to relative tHb increased the adj R^2 from 0.657 to 0.718. Likewise, for absolute VO_{2max} , adding S_aO_2 to absolute tHb increased the adj R^2 from 0.856 to 0.900. Differences in the adj R^2 for absolute versus relative models is most likely attributed to the differences in lean mass of subjects. By adding body mass into the model, we are introducing a greater variability into the measures, which manifests itself as a lower adj R^2 value. As mentioned before, we found that ΔR^2 was not significantly different from zero at our current sample size of six. This means that with our current data, we did not find a statistically significant increase in adj R^2 after adding S_aO_2 into the VO_{2max} -tHb model. However, after running a simulation using our current sample size and correlation, we determined that even if the correlation coefficient drops by 10% by the end of the study ($n=16$), S_aO_2 will still provide a statistically significant contribution in the VO_{2max} -tHb model. For this reason, and

keeping in mind that our data is in line with other reports, we are confident in stating that overall, S_aO_2 has some meaningful effect on VO_{2max} above and beyond tHb. Ultimately, we will be able to analyze this data to get a better idea of how much of an effect S_aO_2 has on the VO_{2max} -tHb model. Regardless of the sample size issue and despite only small changes in the coefficient of determination between models, these observed differences almost certainly represent meaningful physiological variability due to changes in whole body oxygen carrying capability during exercise. Since VO_{2max} is highly dependent on rate of oxygen delivery to the muscles, it is logical that accounting for two variables that have a high influence on rate of oxygen delivery (S_aO_2 and tHb) as opposed to just one (tHb) increased the adjusted coefficient of determination for this model in populations that experience EIAD.

This finding has important ramifications. Prior to this, S_aO_2 and tHb have only been studied separately to assess how each influences endurance performance. In terms of the oxygen transport cascade, the fact that S_aO_2 and tHb interact to influence oxygen transport seems intuitive; this theory clearly separates the movement of oxygen from the air to the arterial blood from the movement of oxygen from the arterial blood to the working muscle. The result of this investigation is a physiological indicator that supports this concept. As a result, when tHb and S_aO_2 are combined, each independently explain some of the variation in VO_{2max} . These results show how the concept of the whole body oxygen carrying capability is physiologically important in regards to VO_{2max} .

Additionally, we found no correlation between tHb, end exercise S_aO_2 , and TTE at PPO. We believe that this is a result of the inherent nature of this performance task. This result implies (and was confirmed via subsequent statistical analysis) that VO_{2max} does not influence TTE. This can be thought of in the following way. VO_{2max} essentially determines PPO, ignoring effects of economy. However, once PPO is determined, other factors influence performance at this workload. We believe that the primary cause of variation in this performance task was due to differences in the non-oxidative capacity of the subjects, which we did not explore in the current investigation.

Limitations/Considerations

It is somewhat of a surprise that prior to this point S_aO_2 , tHb and VO_{2max} had never been examined together given how physiologically important S_aO_2 is in this model. This is likely due to the fact that in the general population at sea level, the majority of people are not expected to experience EIAD, and therefore it would likely be impossible to find a significant increase in correlation in this population. However, we have shown not only that at moderate altitude a majority of healthy but not highly trained males experience EIAD, but also that this decrease in S_aO_2 helps to explain variability in the relationship between tHb and VO_{2max} . However, as of this writing, the sample size of 6 is a major limitation to the generalizability of these results. The low sample size also significantly decreases the

statistical power of our results, and so despite the fact that all but one of our regression analyses (relative tHb and end exercise S_aO_2 versus relative VO_{2max} : $p = 0.069$) were significant at the $p < 0.05$ level, we have to be careful drawing concrete conclusions before all of our data is collected. Additionally, we only studied men around a very narrow age range. This brings up the point that further research is needed in this area to be able to generalize as to the effects of whole body oxygen carrying capability on VO_{2max} across the population. Important populations to study include not only highly trained athletes that are expected to experience EIAD, but also populations where the prevalence of EIAD is likely but less established. For example, it has been shown that women (both aerobically trained and untrained) have higher prevalence of EIAD than equally fit men, due in large part to mechanical ventilatory constraints stemming from smaller lung volumes (Dominelli et al. 2013), and so future studies should look at women of all cardiovascular fitness levels to assess the effects of EIAD and tHb on VO_{2max} in this population. Additionally, other physiological conditions such as exercise induced asthma, vocal cord dysfunction, or chronic obstructive pulmonary disease that have a high likelihood of increasing EIAD during exercise would also provide interesting subject populations for future studies. One additional interesting population to study might be chronic smokers, who due to chronically elevated carboxyhemoglobin levels often have higher than normal tHb and [Hb] (McAloon et al. 1980), but decreased pulmonary function (Anthonisen et al. 2002).

Conclusions

We showed that at moderate altitude 4 of 6 recreationally active subjects experienced either mild ($>3\%$) or moderate ($>4\%$) EIAD. Additionally, since S_aO_2 and tHb have a high influence on rate of oxygen delivery, accounting for S_aO_2 and tHb instead of just tHb decreases the unexplained variability in VO_{2max} . At the current sample size, ΔR^2 was not significantly greater than zero. However, using our current point estimates as population parameters, at a sample size of 12, ΔR^2 will be statistically significant. We conclude that additional studies should be conducted to analyze the influence of whole body oxygen carrying capability on VO_{2max} across the population.

Acknowledgements

This research was funded in part by a grant from the University of Colorado's Undergraduate Research Opportunities Program. This research would not have been possible without the incredible mentoring of both Dr. William Byrnes and Benjamin Ryan. Additionally, the author would like to thank Dr. David Sherwood and Dr. Ray Littlejohn for being on the honors committee.

VO_{2max} ($L \cdot min^{-1}$)	3.8 ± 0.4
Relative VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	49.8 ± 5.9
Peak Power Output during GXT	258.9 ± 31.9
Power at LT (W)	175.0 ± 29.8
VO_2 at LT ($L \cdot min^{-1}$)	2.4 ± 0.4
% VO_{2max} at LT	$64 \pm 5 \%$
Power at LT (W/kg)	2.3 ± 0.2
Economy at LT ($W \cdot L^{-1} \cdot min^{-1}$)	72.3 ± 3.2
Average TTE (seconds)	500 ± 123
Table 1: Selected Endurance Performance Parameters (n = 8). Values are given as Mean \pm SD.	

Subject	Total Hemoglobin Mass		GXT S_aO_2			Average TTE S_aO_2			VO_{2max}	
	Absolute (grams)	Relative (grams/kg)	Resting S_aO_2 (%)	End S_aO_2 (%)	Desaturation (%)	Resting S_aO_2 (%)	End S_aO_2 (%)	Desaturation (%)	Absolute (L/min)	Relative ($L \cdot min^{-1} \cdot kg^{-1}$)
1	932	11.48							3.7	44.7
2	1091	12.54							3.6	41.1
3	1005	12.27	98.5	97.1	1.5	98.9	96.1	2.8	4.3	52.3
4	840	11.92	97.0	93.8	3.3	98.4	94.8	3.6	3.3	46.4
5	1135	12.69	94.0	91.5	2.5	96.3	93.3	3.0	4.5	50.3
6	789	13.89	100.0	94.0	6.0	100.0	95.0	5.0	3.4	59.2
7	852	11.48	98.0	94.0	4.0	98.0	92.0	6.0	3.6	49.0
8	992	14.39	97.3	92.5	4.8	96.9	93.6	3.3	3.8	55.7
Average \pm SD	954.5 ± 123	12.58 ± 1.1	97.5 ± 2.0	$93.8 \pm 1.9^*$	3.7 ± 1.6	98.1 ± 1.4	$94.1 \pm 1.5^*$	3.9 ± 1.3	3.8 ± 0.4	49.8 ± 5.9

Table 2: Total Hemoglobin Mass Data (n = 8) and S_aO_2 data (n = 6). tHb data represents the average of two measurements except for subjects 3 and 5, where only one measurement was used. S_aO_2 data measured using forehead pulse oximetry. Data was collected every 15 seconds for one minute during rest, and averaged over the final of exercise (n = 6). * $p < 0.05$ different from rest. VO_{2max}

<i>Model</i>	<i>R</i>	<i>R²</i>	<i>adj R²</i>	<i>p value</i>	<i>slope</i>	<i>Reported Slope</i>	<i>Reported r²</i>
<i>Dependent variable (Independent variables)</i>							
Absolute							
VO2max (tHb) ^a	0.91	0.88	0.86	0.005	3.56	4.4	0.94
VO2max (tHb, end GXT saturation) ^b	0.97	0.94	0.90	0.015			
Change between models		$\Delta = 0.06$		0.196			
Relative							
VO2max (tHb) ^c	0.85	0.73	0.66	0.031	3.48	3.4	0.65
VO2max, (tHb, End GXT Saturation) ^d	0.91	0.83	0.72	0.069			
Change between models		$\Delta = 0.10$		0.265			

Table 3: Effects on metrics related to the best fit from 4 separate models (n = 6). The reported slope and r² is from Schmidt and Prommer (2010) and is based on linear regression analysis of tHb and VO_{2max}. p value for ΔR^2 represents the probability that the change in R² from adding S_aO₂ is statistically significant (^a - figure 3, ^b - figure 4, ^c – figure 5, ^d – figure 6).

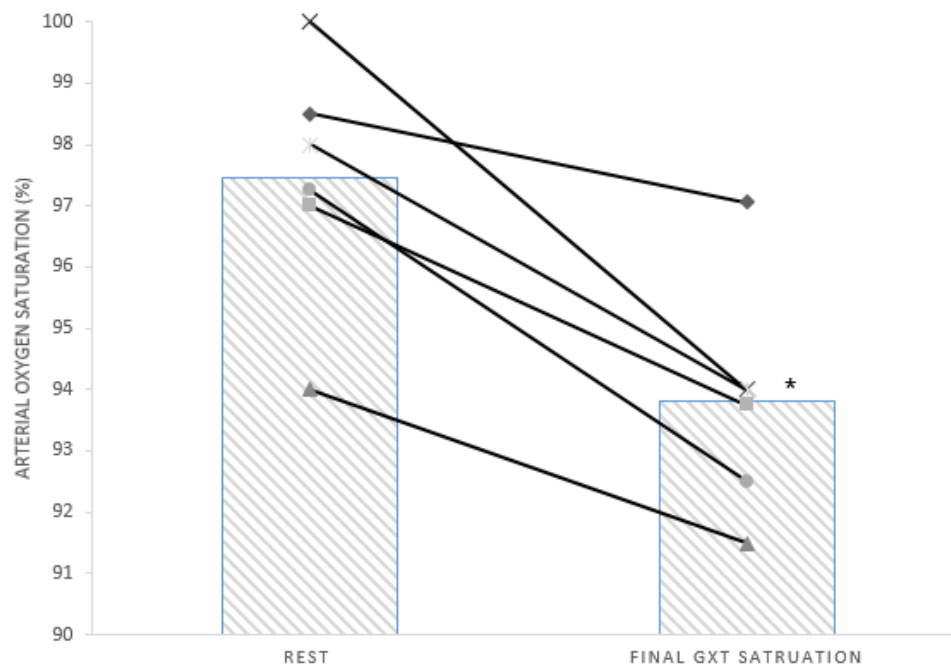


Figure 1: Arterial oxygen saturation at rest and during the final minute of a GXT for 6 individual subjects. Lines represent individuals, bars represent the average. * significantly different from rest, $p < 0.05$.

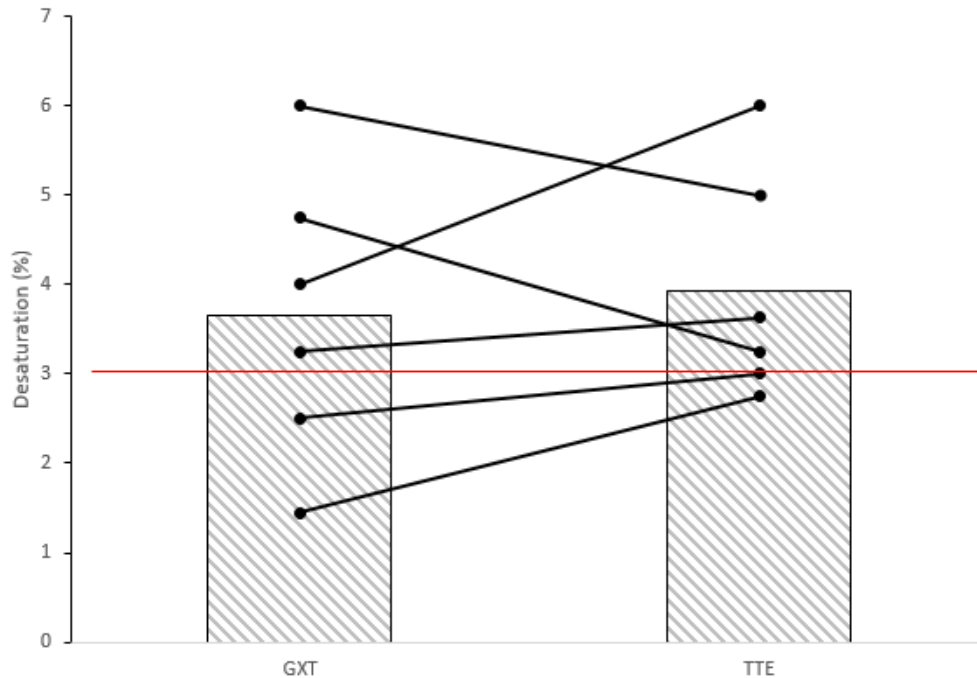


Figure 2: Intrasubject variability between desaturation during GXT versus TTE. Line across center represents 3% desaturation. Subjects above the line experienced EIAD, while those below did not. Bars represent the average. No significant difference was found between GXT and TTE desaturation.

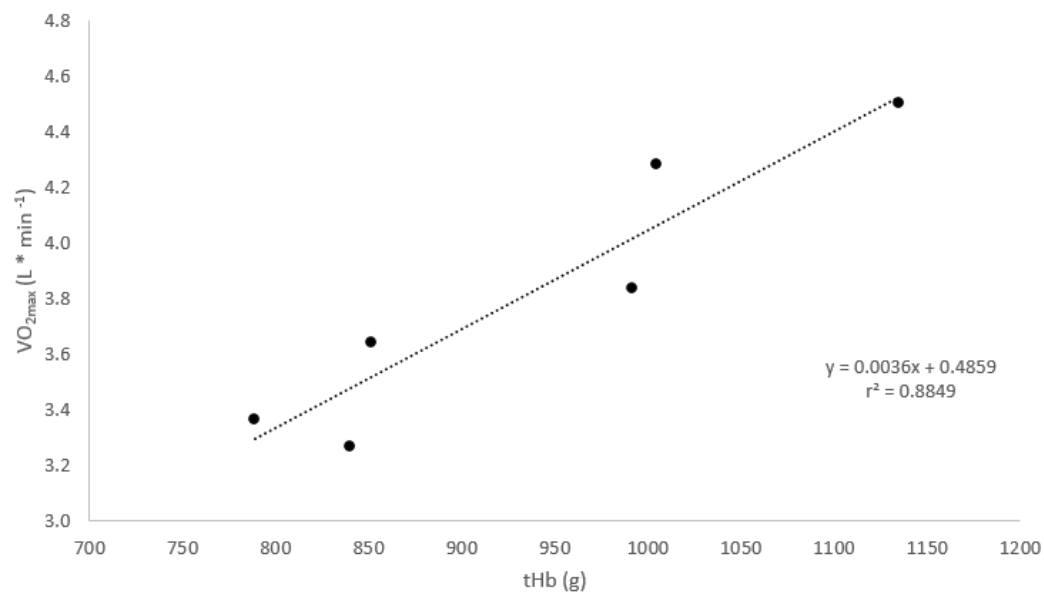


Figure 3: tHb vs. VO_{2max}. adj $r^2 = 0.86$, $r^2 = 0.88$

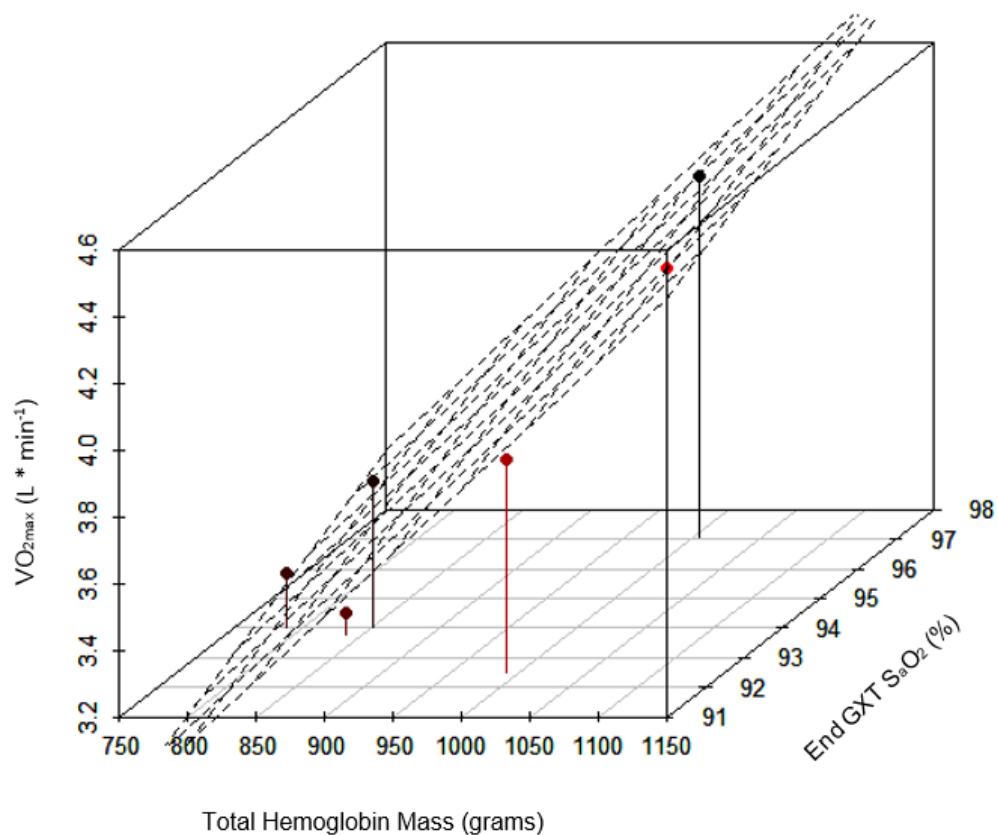


Figure 4: Plot of 3 dimensional regression analysis with saturation and tHb versus VO_{2max}. adj $R^2 = 0.90$, $R^2 = 0.94$

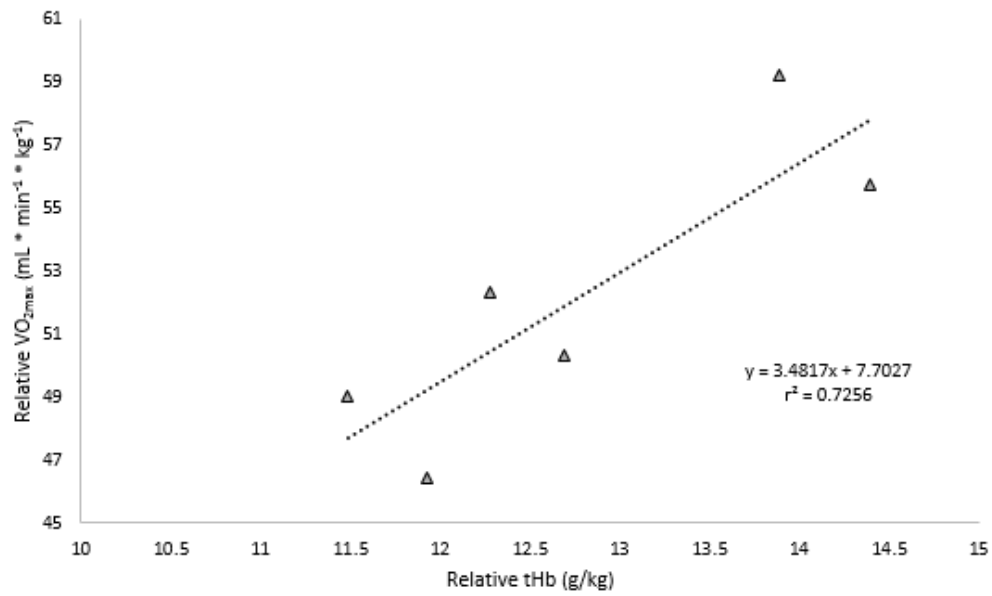


Figure 5: Relative tHb vs. relative VO_{2max}. adj $r^2 = 0.66$, $r^2 = 0.73$

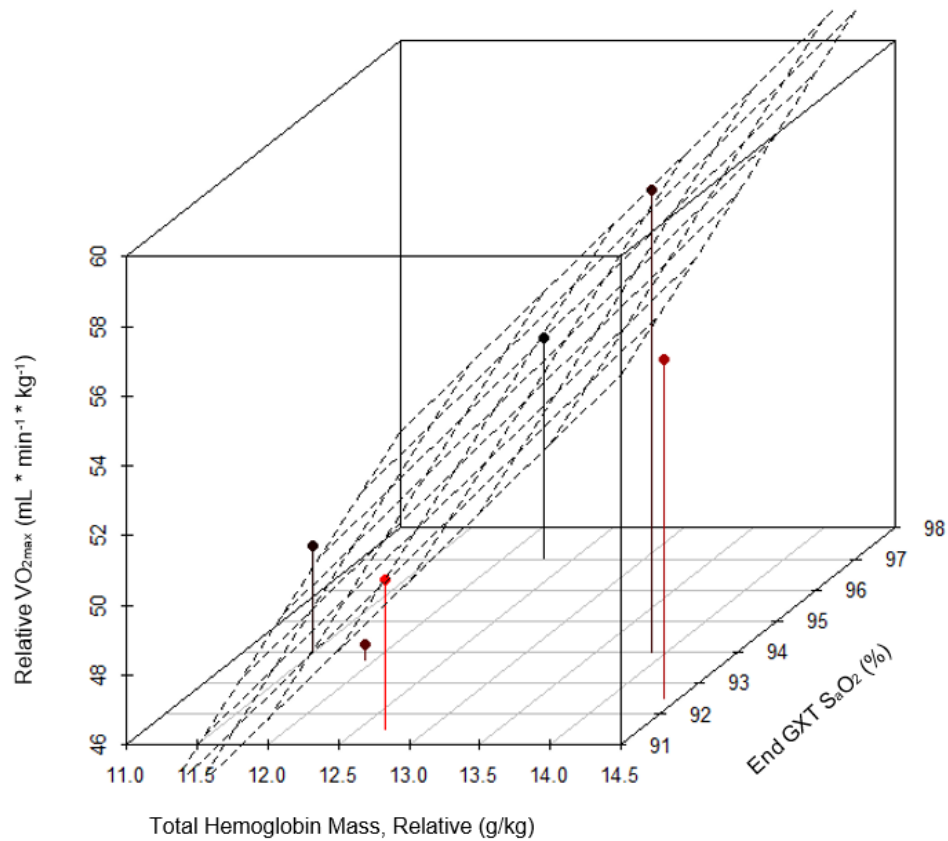


Figure 6: Plot of 3 dimensional regression analysis with saturation and relative tHb versus relative VO_{2max}. adj $R^2 = 0.72$, $R^2 = 0.83$

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